MICROBIOLOGY AND TECHNOLOGY OF THE PEPPERONI PROCESS

ABSTRACT

A pilot plant process was developed for the production of pepperoni as a fully dry, fermented sausage. The process included: (a) aging in which salted (3% NaCl) meat was held for 10 days at 5°C to encourage the growth of micrococci and lactobacilli; (b) fermentation at 35°C and 85% relative humidity (RH) during which the lactobacilli fermented the sugar and lowered the pH, and the micrococci reduced nitrate to nitrite; and (c) drying at 12°C and 65% RH to about 50% of starting weight. Chemical analyses of the commercial pepperoni yielded the following data: (1) pH ranged from 6.1–4.7; (2) moisture, from 17.0–30.9%; (3) fat, from 38.1–52.5%; (4) water activities ranged from 0.87–0.80; and (5) all had moisture/protein (M/P) ratios < 1.6/1.0, the maximum recommended for pepperoni. The microflora of commercial samples varied, both in bacterial count and type. Pilot plant products had lower moisture and fat contents than commercial products, pH values of 4.7–4.9, and viable microflora almost exclusively lactobacilli. The M/P ratios of the pilot plant products were also < 1.6/1.0.

INTRODUCTION

PEPPERONI is a highly spiced (with or without paprika), fermented, fully-dry sausage prepared from pork or a mixture of pork and beef. Except for the drying step, its processing should be similar to that of other fermented sausages such as Lebanon bologna or summer sausage. However, neither details of the process nor the chemical and microbiological changes which occur during the various steps have been reported in the literature. We wanted to develop a process which would yield sausages with characteristics similar to those of commercial pepperoni and to follow the chemical and microbiological changes throughout processing. We also analyzed commercial pepperoni chemically and microbiologically.

MATERIALS & METHODS

Sugars-spices-cure

Based on published formulae (Kramlich et al., 1973; Komarik et al., 1974; National Provisioner, 1938), the following sugar/spice mixture was developed for our pepperoni:

Sugar/spice	g/kg meat mix
sucrose	10
glucose	10
ground cayenne pepper	2
crushed red pepper	2
pimento	5
whole anise seed	2-1/2
garlic powder	0.1

The curing agent, $NaNO_3$, was added at 1.2 g/kg of meat. This level was a compromise between the 0.6 g/kg recommended by Kramlich et al. (1973) and the ca 1.7 g/kg permitted by Meat Inspection Regulations (Meat and Poultry Inspection Program, APHIS, USDA, 1973, Washington, D.C.).

Meat

Boneless pork shoulders or picnics were ground through a 3/4-in. plate and frozen at −27°C for at least 1 month to inactivate the trichinae. Whole, good-grade beef chuck was removed from the carcass, ground through a 3/4-in. plate, frozen at −27°C and held until needed. Prior to use, meats were thawed at 10°C.

Fermentation

Pilot plant pepperoni was fermented either by the natural lactic microflora of the meat encouraged through aging of salted (3% NaCl) meat at 5°C for 10 days (Palumbo et al., 1973; Smith and Palumbo, 1973) or in one experiment, by the addition of lactic acid starter culture (Lactacel MC, Merck & Co., Rahway, N.J.).

Compositional and chemical analyses

Moisture, ash, fat and protein contents of the various pepperoni were determined on twice-ground (1/8-in. plate) samples by standard AOAC procedures (AOAC, 1965). The ground pepperoni samples were analyzed for nitrosamines by methods described previously (Palumbo et al., 1974). The pH and titratable acidity were measured by a modification of the technique of Kempton and Bobier (1970); 50g of the salted meat sample or of intact sausage were aseptically weighed into a sterile Waring Blendor jar; 200 ml of sterile 0.1% peptone (Difco) water were added, and the mixture was blended for 1 min at high speed. The resulting slurry (1:5 dilution) was first sampled for microbiological analyses, then centrifuged at 5°C for 10 min at 16,500 × G. The pH of the clear extract was measured on a Corning model 10 pH meter using a single, combination electrode. The acid present in the extract (generally a 50 ml aliquot) was titrated to pH 7.0 by using the pH meter and standard base; percent acid was calculated as lactic. Water activity (A_w) was determined on diced slices of pepperoni by use of an Electric Hygrometer-Indicator (model 15-3001, with gray sensor; Hydrodynamics Inc., Silver Spring, Md.).

Microbiology

Microbiological analyses of commercial pepperoni were performed; the microflora of our own pepperoni was determined during aging of the meat and during and after processing of the sausages. The peptone water slurry was used for microbial analyses as follows (Smith and Palumbo, 1973): total count on APT agar (Difco) incubated for 3–4 days at 25°C; micrococci (gram-positive, catalase-positive cocci) on Mannitol salt agar (MSA, Difco) incubated for 3 days at 32°C; lactobacilli (gram-positive, catalase-negative rods) on Rogosa SL agar (Rog, Difco) incubated for 3–5 days at 25°C; yeast on acidified potato dextrose agar (PD, Difco) incubated for 3 days at 25°C; and gram-negative rods (coliforms) on Eosin Methylene Blue agar (EMB, Difco) incubated for 1 day at 37°C. Gram stain and catalase test were performed on all colony types found on the various media.

Processing

The following general procedure was developed in our pilot plant for pepperoni: The frozen meat was thawed and mixed with 3% salt (NaCl); the salted meat was then aged for ca 10 days (the aging step was omitted when starter culture was used). After aging, the sugar-spice mixture and $NaNO_3$ were added to the meat, which consisted of either pork or beef or a 1:1 mixture of the two, and mixed. This mixture was then ground through a 3/16-in. plate, stuffed into 55 mm clear fibrous casings (Union Carbide), coated with paraffin (MP, 52°C), and hung at 35°C and 85% RH for 1–3 days to allow fermentation. The paraffin which was used to prevent mold growth and excessive moisture loss during fermentation was removed after fermentation and the sausages were dried for 6 wk (40–42 days) at 12°C and 65% RH.

RESULTS & DISCUSSION

SEVERAL DIFFERENT commercial pepperoni were purchased and analyzed chemically and microbiologically. All were prepared from a pork and beef mixture, most with a mixed (nitrate/nitrite) cure; ca 1/2 contained paprika, and ca 1/3 were fermented with starter culture (Table 1).

Compositional and chemical evaluations of commercial and

pilot plant pepperoni are given in Table 2. The fat content of commercial products ranged from 38.1–52.8%. The protein varied from 17.9–24.8%, ash from 5.1–6.4 %, and moisture from 17.0–31.5%. For all commercial and pilot plant pepperoni, moisture/protein ratios (M/P) were <1.6/1.0, the maximum permitted for pepperoni in the Laboratory Guide Book (USDA, CMS, Laboratory Services Div.).

The pH values for commercial pepperoni (Table 2) varied widely, ranging from 6.1–4.8. Usually commercial products with low pH had high titratable acidity. With one exception, from company D, only commercial products prepared with starter culture had pH values <5.0 (Tables 1 and 2). Though Lechowich (1971) stated that pH 4.5–5.0 is desirable in fermented sausages, six commercial pepperoni had pH > 5.0 and five, <5.0. Apparently other commercial fermented sausages also exceed pH 5.0. Ostlund and Regner (1968) recorded a pH range of 5.7–4.6 for commercial samples of "Isterband," a Swedish fermented sausage. Deibel et al. (1961) found a pH range of 5.3–4.6 for commercial summer sausage, but a very narrow range of 4.9–5.1 for thuringer. In contrast, commercial Lebanon bologna had a narrow, desirable pH range of 4.6–4.9 (Palumbo et al., 1973). After drying, our pepperoni had pH values of 4.7–4.9 and titratable acidities of 1.14–1.54% (Table 2).

In general, agreement was good among moisture contents, M/P ratios, and A_w values (Table 2). Commercial pepperoni having high moisture contents tended to have high M/P ratios and A_w values.

The microbiological analyses of commercial pepperoni are given in Tables 3 and 4. Except for the total count (APT agar), the counts on the other media varied (Table 3), from undetectable ($<1 \times 10^2$/g) to substantial. For example, on Rog, the counts ranged from 1×10^2/g for company E to 8.5×10^7/g for company F (loc 1). Data in Table 4 indicated that the selective agars used to examine samples of commercial pepperoni supported the growth of several types of microorganisms in addition to those the agars are supposedly designed to detect and distinguish. Virtually all organisms isolated from these sausages grew on MSA (Table 4). Thus, our

Table 1—Ingredients of commercial pepperoni[a]

Company[c]	Components added to meat cure[b]			
	Paprika	Nitrate	Nitrite	Added starter culture
A	–	+	–	–
B	–	1	2	–
C	–	1	2	–
D	–	1	2	–
E	+	1	2	–
F (loc 1)[c]	+	–	+	+
F (loc 2)[c]	+	1	2	+
F (loc 2)[c] sandwich style	+	1	2	+
G	+	1	2	–
H	–	2	1	+
Imported	–	–	+	–

[a] From their respective lists of ingredients. All commercial products were prepared from a mixture of pork and beef.
[b] A + indicates the presence of that particular curing agent; where both were employed, the one listed first on the ingredient list is indicated by a 1 and the one listed second is indicated by a 2.
[c] The products of company F are manufactured at two plants, designated loc 1 and loc 2. Sandwich style designates a product produced in a wide (ca 45 mm finished size) casing; all other products were of the stick variety, finished diameter ca 25 mm.

Table 2—Compositional analyses and chemical measurements of commercial and pilot plant pepperoni

Company	Moisture (M), (%)	Ash (%)	Fat (%)	Protein (P), (%)	M/P Ratio	A_w	pH	% Acid as lactic
				Commercial pepperoni				
A	30.9	5.4	39.2	20.2	1.48	0.87	5.5	0.49
B	17.0	5.6	50.0	21.2	0.80	0.80	5.2	0.71
C	17.0	5.3	52.8	20.1	0.85	0.81	6.1	0.29
D	31.5	6.4	38.1	20.5	1.54	0.83	4.8	0.84
E	22.6	6.1	44.1	21.5	1.05	0.84	5.8	0.40
F (loc 1)[a]	30.8	5.5	41.7	21.1	1.46	0.87	4.9	0.56
F (loc 2)[a]	27.1	5.8	43.7	24.8	1.09	0.84	4.9	0.61
F (loc 2)[a] sandwich style	26.3	5.4	47.7	20.4	1.29	0.85	4.8	0.65
G	25.4	5.6	42.2	20.8	1.22	0.86	5.1	0.55
H	18.4	5.4	52.5	21.9	0.84	0.81	4.7	0.73
Imported	21.1	5.1	51.0	17.9	1.18	0.83	5.3	0.37
				Pilot plant pepperoni				
Expt. I (all pork)	24.1	6.3	37.5	28.3	0.85	0.81	4.7	not done
Expt. II (all pork)	20.8	6.7	40.6	28.6	0.73	0.80	4.8	1.14
Expt. III								
all beef	28.9	7.9	18.1	41.3	0.70	0.82	4.9	1.54
all pork	27.0	7.1	32.2	32.2	0.84	0.83	4.8	1.42
pork-beef	28.6	7.6	23.0	37.7	0.76	0.82	4.8	1.33

[a] The products of company F are manufactured at two plants, designated loc 1 and loc 2. Sandwich style designates a product produced in a wide (ca 45 mm finished size) casing; all other products were of the stick variety, finished diameter ca 25 mm.

Table 3—Number of viable microorganisms present in selected commercial pepperoni

Company	Viable counts per gram of pepperoni plated on[a]				
	APT	PD	Rog	EMB	MSA
A	1.3×10^8	3.3×10^3	3.7×10^7	1.0×10^5	1.2×10^7
B	1.0×10^4	$< 1 \times 10^2$	1.0×10^2	3.0×10^2	$< 1 \times 10^2$
C	2.5×10^7	9.6×10^3	1.4×10^5	3.0×10^5	1.1×10^7
D	2.3×10^7	1.0×10^5	4.0×10^4	$< 1 \times 10^2$	5.0×10^4
E	6.0×10^6	1.0×10^2	$< 1 \times 10^2$	3.5×10^2	1.5×10^5
F(loc 1)[b]	1.0×10^8	$< 1 \times 10^2$	8.5×10^7	$< 1 \times 10^2$	2.7×10^3
F(loc 2)[b]	3.3×10^6	1.0×10^2	1.6×10^6	$< 1 \times 10^2$	2.0×10^3
F(loc 2)[b] sandwich style	1.0×10^4	$< 1 \times 10^2$	2.7×10^3	3.9×10^3	1.1×10^4
G	7.0×10^6	1.1×10^5	6.9×10^5	3.0×10^4	1.3×10^6
H	1.2×10^7	1.6×10^4	6.0×10^6	1.8×10^3	7.0×10^3
Imported	2.5×10^6	$< 1 \times 10^2$	1.1×10^6	1.0×10^4	1.7×10^6

[a] APT, APT agar; PD, acidified potato dextrose agar; Rog, Rogosa SL agar; EMB, Eosin Methylene Blue agar; MSA, mannitol salt agar.
[b] The products of company F are manufactured at two plants, designated loc 1 and loc 2. Sandwich style designates a product produced in a wide (ca 45 mm finished size) casing; all other products were of the stick variety, finished diameter ca 25 mm.

practice of examining the gram and catalase reaction of all colony types is an effective and necessary method of studying the microflora of commercial sausages.

Process

After examination of commercial pepperoni, we sought to produce a fully-dry sausage with characteristics similar to those of the commercial products. Using the microbiology and technology for Lebanon bologna (Palumbo et al., 1973; Smith and Palumbo, 1973) and some published recipes (Kramlich et al., 1973; Komarik et al., 1974; National Provisioner, 1938), we devised a process consisting of periods of aging, fermentation, and drying. The microbiological and chemical changes were determined during the three periods. Although the data of single experiments are reported in Figures 1–5, all experiments were repeated at least twice and patterns were similar in all instances.

Table 4—Cellular types, and gram and catalase reactions of the microflora found in commercial pepperoni

Company	Microbial types[a] found on the following media				
	APT	PD	Rog	EMB	MSA
A	a	e	a	f	c
B	b,d	—	f	c	—
C	c	e	a	c	c
D	a	e	e	—	e
E	c,b	e	—	d	c
F(loc 1)[b]	f	—	f	—	f
F(loc 2)[b]	c	e	c	—	b
F(loc 2)[b] sandwich style	b	—	a	b	b,d
G	a,c	e	a	f	c
H	f	e	f	b	b,e
Imported	a	—	a	f	c

[a] a = lactobacilli (catalase-negative, gram-positive rods).
b = bacilli (catalase-positive, gram-positive sporeforming rods).
c = micrococci (catalase-positive, gram-positive cocci).
d = catalase-positive, gram-negative rods (not typical coliforms).
e = yeast.
f = catalase-negative, gram-positive cocci.
[b] The products of company F are manufactured at two plants, designated loc 1 and loc 2. Sandwich style designates a product produced in a wide (ca 45 mm finished size) casing; all other products were of the stick variety, finished diameter ca 25 mm.

Microflora during aging

We had examined the microflora of beef during aging (Smith and Palumbo, 1973), but we repeated that earlier study. Pork was included because of its high thiamine content (0.76 mg/100g pork vs 0.06 mg/100g beef; Rice, 1971) and thus might support more rapid development of lactobacilli than beef. Changes in the microflora of aging beef and pork are presented in Figure 1. The selective agars (PD, Rog and MSA) were extremely useful in defining the microbial types developing during aging of meats for our pilot plant pepperoni. This usefulness may be attributed to the fact that we have meats with low background flora and few contaminating bacteria. During aging, PD supported only yeasts, Rog only lactobacilli, and MSA only micrococci. However, gram-positive, catalase-positive rods (bacilli) were recovered on EMB which was used to detect the presence of coliforms. No coliforms were detected in any pepperoni or meat sample, and therefore, EMB counts have not been given. Unexpectedly, the Rog count increased faster in beef than in pork, while the MSA count was just the opposite. The basis for this is unknown, but it could be theorized that the growth of lactobacilli in pork was limited by some component other than thiamine and that specific nutrient content of beef favored lactobacilli and that of pork micrococci. The number of lactobacilli which developed naturally during aging of salted beef and pork were sufficient to carry out the fermentation and the reduction of nitrate to nitrite (Smith and Palumbo, 1973).

Fermentation

The changes in the microflora during fermentation of pilot plant pepperoni are given in Figure 2. Again the selective agars were extremely useful in defining the microbial sequence during the fermentation: the yeasts died off and were not detected after the first day of fermentation; only lactobacilli were found on Rog, and micrococci on MSA. In general, the counts for an all-pork and a pork-beef pepperoni were similar. Data were also similar for an all-beef pepperoni.

The most important change during the fermentation was the conversion of the sugars to lactic acid. During the 3-day fermentation of a pork-beef pepperoni, pH decreased from 6.3 to 4.7, while titratable acidity increased from 0.23 to 0.63%.

Data were similar for all-beef and all-pork pepperoni. Our pilot plant pepperoni contained as much acid after fermentation as the samples of commercial product did after drying (compare Fig. 3 with Table 2).

Drying

During drying for 6 wk (ca 40–42 days), the pepperoni lost ca 50% of their "green" (starting) weight and took on the characteristic appearance of this sausage type; their A_w decreased to 0.82 (Fig. 4). The 50% weight loss classed products as fully dry sausage (MacKenzie, 1966); M/P <1.6/1.0 also define the pilot plant pepperoni and comm products as fully dry. Fermented sausages may also be according to their final moisture content; a final moistu tent of 35% or less designates all pepperoni examined a dry (Kramlich, 1971). The decreases in A_w and weigh pork-beef pepperoni were similar to those for the all-po the all-beef pepperoni.

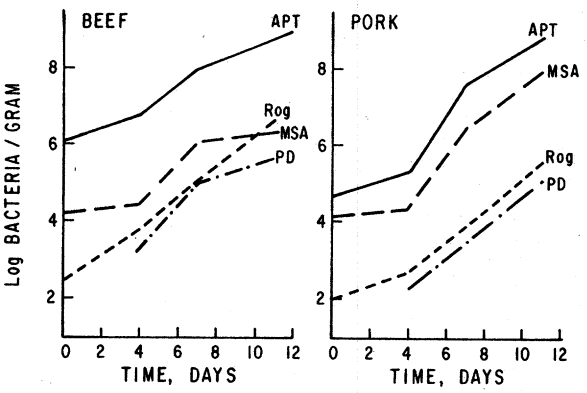

Fig. 1—Changes in microflora plated on various media during aging of salted (3% NaCl) beef and pork at 5°C (see Materials & Methods for media designations).

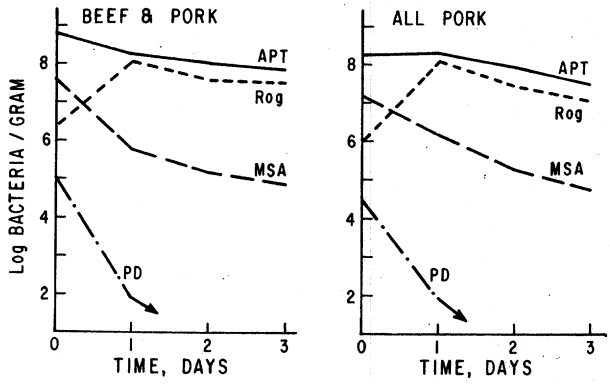

Fig. 2—Changes in microflora plated on various media during the fermentation of a pork-beef and an all-pork pepperoni at 35°C (see Materials & Methods for media designations).

Figure 4 shows that the sausages lost moisture rapidly in the drying period, but the A_w did not change much suggested that early in drying, large amounts of moisture be lost to yield small changes in A_w. Townsend and (1972) found that Genoa salami weight losses were g early in drying, and decreased as the 21-day drying ended. Skjelkvole et al. (1974) observed that A_w in Nor salami changed little during the early part of the ri (drying), and decreased substantially only toward th Wardlaw et al. (1973), who followed moisture content o mer sausage during 60 days' drying, found a pattern sim that in Figure 4. Thus, the moisture changes in our pep were similar to those reported for other sausage types drying.

During drying of the pepperoni, some portions microbial population remained constant while othe creased (Fig. 5). For both the pork-beef and the a pepperoni, the total (APT agar) and Rog counts remaine stant during the 40-day drying period. On Rog and AP lactobacilli were detected. The extended viability of bacilli observed in our pilot plant pepperoni is consister the high lactobacilli counts (APT and Rog) we found i ples of commercial pepperoni (Tables 3 and 4).

The count observed on MSA showed little change drying. However, at 19 days (see arrow on Fig. 5), the isms found on MSA changed from micrococci to bacilli positive, catalase-positive sporeforming rods) and no micrococci could be detected during the rest of the period. Micrococci were detected in only 6 of the comm products, generally in those with pH values above 5.(decline of micrococci to undetectable levels was also ob in Lebanon bologna (Smith and Palumbo, 1973). Af 4-day Lebanon bologna fermentation, no micrococci 10^2/g) were detected. Smith and Palumbo (1973) th that the acid content of Lebanon bologna was responsi the decline of viable micrococci. Further support of th sensitivity is now offered. On the 19th day of drying the acid content of the pepperoni described in Fi reached 1%, the micrococci had become undetectable. other experiment (data not presented), all-beef peppero taining different levels of fat were fermented for only and then dried. Because of the short fermentation tin

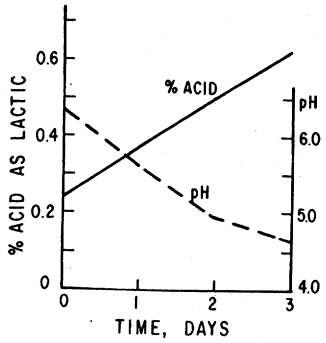

Fig. 3—Changes in pH and % acid (as lactic) during the fermentation of a pork-beef pepperoni at 35°C.

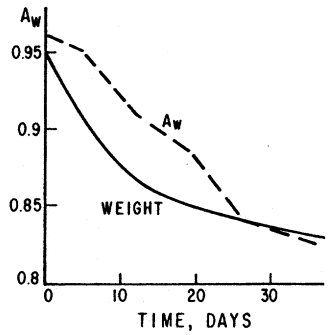

Fig. 4—Changes in moisture relationships of a pork-beef pepperoni during drying at 12°C and 65% relative humidity.

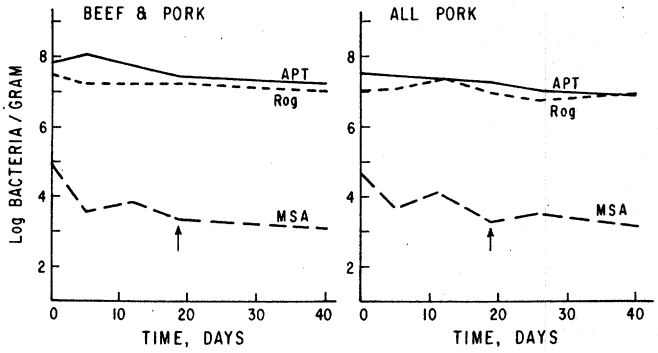

Fig. 5—Changes in microflora during drying of a pork-beef and all-pork pepperoni at 12°C and 65% relative humidity (see Materials & Methods for media designations).

acid was produced so that, during the drying period, the acid content of these all-beef pepperoni never reached the 1% level, and micrococci could be detected throughout the entire drying period. Furthermore, Lebanon bologna produced in our pilot plant had final acid contents substantially above 1% (Palumbo et al., 1973) and no micrococci were detected after the 4th day of fermentation (Smith and Palumbo, 1973). Among commercial Lebanon bolognas, those with higher acid contents generally did not have detectable levels ($<1.0 \times 10^2$/g) of micrococci (Palumbo et al., 1973; Smith and Palumbo, 1973).

Lactobacilli and micrococci have remained viable in other dry sausages as reported by Skjelkvole et al. (1974) during the ripening of Norwegian salami, and by De Ketelaere (1974) during the ripening of a dry sausage. However, the pH of their experimental sausages was low enough that the colonies they counted on S110 agar might have been bacilli. They apparently did not use Gram stains which would have clarified this point.

During drying, the pH of our pepperoni remained constant or changed no more than a tenth of a pH unit. However, since the sausages were dehydrated to 50% of their green weight, the final titratable acidity was double the initial.

To complete our study of pepperoni processing, we sought to determine the factor(s) responsible for development of a firm textured sausage during drying. During other studies, we observed that sausage which was not fermented did not yield firm textured products upon drying. However, many commercial products are barely fermented (pH > 5.0, Table 2) and yet have a firm texture. We theorized that possibly a mild heat treatment in the form of a trichina cook (internal temperature of 137–140°F) would permit development of a firm texture during drying of a nonfermented sausage; we designed an experiment to test this hypothesis using pork-beef (1:1) pepperoni. The variables included: nonfermented, pH 5.6; natural flora-fermented, pH 4.8 (aged meat); and starter culture-fermented, pH 4.6. There were both heated and nonheated

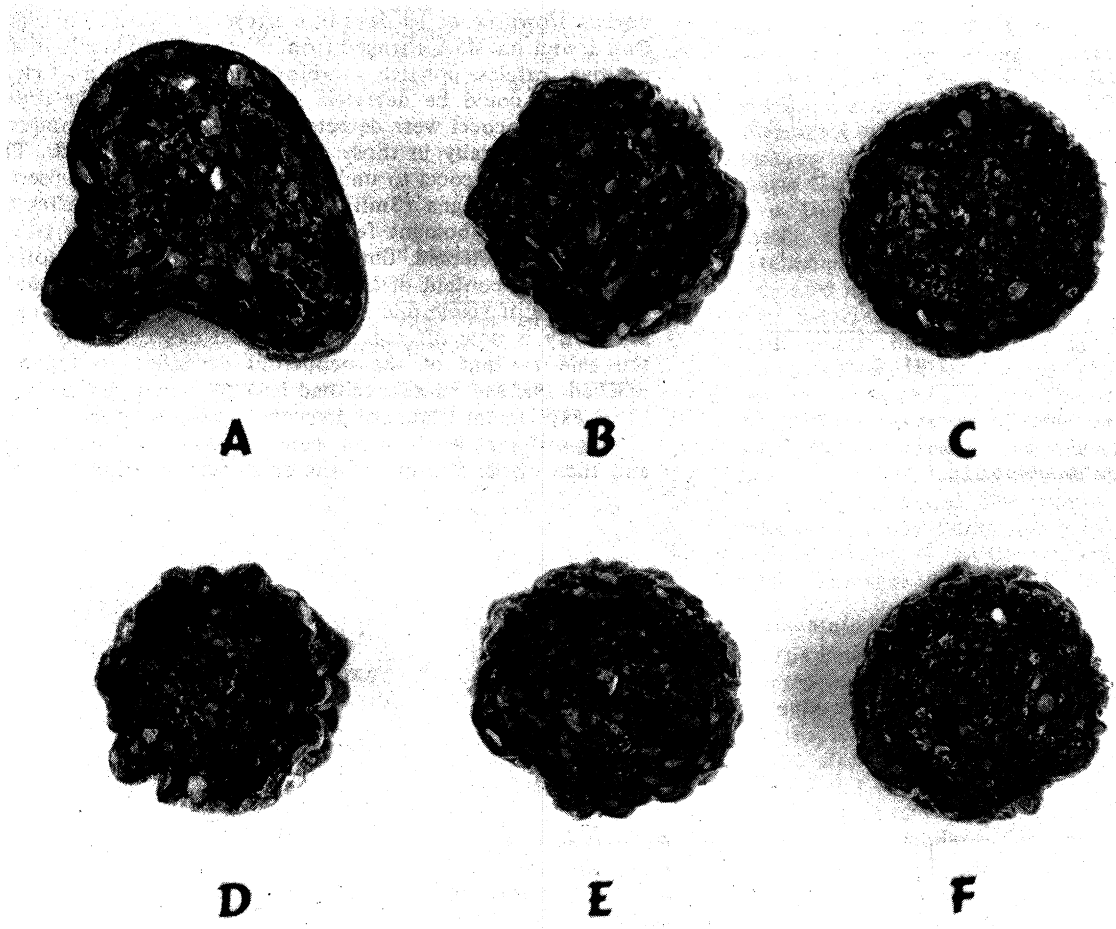

Fig. 6—Photograph of pepperoni slices approx. 1X. A, B, and C were nonheated and D, E, and F were heated to 140°F. A and D were nonfermented (pH 5.6); B and E were natural flora-fermented (pH 4.8); and C and F were starter culture-fermented (pH 4.6).

sausages for each fermentation. All heated sausages, even the nonfermented ones, had firm texture upon drying; with the nonheated sausages, only the fermented ones (natural flora or starter culture) had firm texture upon drying (Fig. 6). The nonfermented, nonheated sausages were misshapen and had hollow centers with poor, grainy texture (Fig. 6). All sausages lost moisture at ca the same rate and had similar final moisture contents. We can conclude from this last experiment that acid formation (fermentation) or heating will yield a firm textured product upon drying.

In a previous study (Palumbo et al., 1974), we investigated the potential for nitrosamine formation during processing of Lebanon bologna, a fermented sausage. Since pepperoni possesses many of the same product characteristics as Lebanon bologna, we investigated the potential for nitrosamine formation during processing of pepperoni. We examined natural flora fermented pork-beef pepperoni, prepared with and without paprika, and found no detectable levels of the 6 volatile nitrosamines for which we tested. One commercial sample examined was also negative for these same six nitrosamines.

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Reference to a brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

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